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MICROCIRCULATORY IMPAIRMENT FOLLOWING FOCAL AND GLOBAL CEREBRAL--ETC(U)
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anti-inflammatory drugs (indomethacin, ibuprofen, flurbiprofen), prostacyclin, antiserum against presumptive factor-VIII antigen, glass-wool filtration of the blood, and alteration of blood pressure. Contrary to previous work in the dog, these interventions tended to blunt or to eliminate the hyperemic response after reversible ischemia. Unilateral cerebral air emboli evoked a global increase in intermediary-carbohydrate metabolites. Serial air emboli caused a stepwise deterioration in CBF that could not be ascribed to stationary intravascular air but that was accelerated by lowered blood pressure.

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FINAL TECHNICAL REPORT

MICROCIRCULATORY IMPAIRMENT FOLLOWING FOCAL AND GLOBAL CEREBRAL ISCHEMIA IN THE RAT

Contract No. N00014-79-C-0771
Office of Naval Research

Dates: 1 August 1979 - 31 October 1981

Principal Investigator: Thomas W. Furlow, Jr., M.D.
Department of Neurology
University of Alabama in Birmingham
Birmingham, Alabama 35294
(205) 934-3866

Accountant For

NAME	DATE
	X

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1.

ABSTRACT

The studies carried out during the two-year tenure of this contract sought to explore certain biochemical influences on the course of blood perfusion of the ischemically injured brain. Two models of reversible cerebral ischemia in the rat were employed, one focal (selective air embolism of one cerebral hemisphere) and the other global (bilateral occlusion of the carotid and vertebral arteries). A 15-min period of cerebral ischemia combined with arterial hypotension (MABP of 50 Torr) was followed by observation for 1 hr. Local cerebral blood flow was measured serially by the polarographic hydrogen-clearance technique and terminally by (14C)-iodoantipyrine autoradiography. Modes of therapeutic intervention investigated included prostaglandin-synthetase inhibitors (indomethacin, ibuprofen, flurbiprofen), prostacyclin, antiserum against presumptive factor-VIII antigen, glass-wool filtration of blood, and alteration of arterial blood pressure.

Contrary to the previous findings in the dog, most of these therapies had a slight depressing effect on terminal blood flow and they tended to blunt or obliterate the hyperemic response that follows reversible ischemia of the brain. Whether this lowering effect on postischemic blood flow was beneficial or detrimental on recovery of neural function was not established. Additional experiments designed to study the effects of unilateral micro-emboli of air on the brain unexpectedly revealed a bilateral enhancement of intermediary-carbohydrate metabolism. Serial emboli of air (2 uL) caused a stepwise deterioration in local cerebral blood flow (1) that could not be attributed to mere blockage of the microcirculation by the air and (2) whose severity was inversely related to MABP.

II.

INTRODUCTION

Previous work by the principal investigator and his collaborators has demonstrated striking modification of postischemic blood flow through the dog's brain by various manipulations¹⁻⁶. Such disparate techniques as glass-wool filtration of blood^{1, 4} or injection of indomethacin with⁵ or without³ prostacyclin infusion enhance local cerebral blood flow after CSF compression ischemia, whereas administration of factor-VIII antigen following glass-wool filtration is detrimental to postischemic blood flow⁶.

Owing to the clinical importance of preserving cerebral blood flow after injury, the present studies were undertaken to determine the universality and molecular basis of such interventions in another experimental animal, the rat. The following technical report summarizes the results of these laboratory investigations.

III.

METHODS

A. Animals:

All rats employed in these investigations were of the Sprague-Dawley line purchased from Charles River Farms or derived from animals of the C-D strain from that source. Spontaneously hypertensive rats of the Okamoto-Aoki line were also purchased from the same supplier. Male animals were used exclusively, and their weights ranged from 250-400 g, although blood drawing for study of factor VIII was limited to heavier retired breeders.

B. Animal Preparation and Monitoring:

All surgical procedures were performed under anesthesia induced by mask delivery of fluothane and oxygen. A Silastic® tracheostomy tube was inserted and polyethylene catheters (PE-50, Clay Adams, Parsippany, NJ 07054) were placed into the femoral arteries and veins. Unless otherwise noted, each rat was paralyzed postoperatively with D-tubocurarine (5 U subcut) and connected to a rodent ventilator (model 680, Harvard Apparatus, Millis, MA 02054) with oxygen supplementation to keep an arterial oxygen tension in excess of 90 Torr. The arterial tension of carbon dioxide was held to 35 ± 5 Torr whenever possible, and the arterial pH was maintained between 7.35 and 7.40. Blood gases were serially measured from 100-uL samples of arterial blood (Micro 13-01/213-05, Instrumentation Laboratory, Lexington, MA 02173). Body temperature was maintained at $37.50 \pm 0.50^\circ\text{C}$ with a heat lamp regulated by a temperature controller (model 73 ATF, Yellow Springs Instruments, Yellow Springs, OH 45387) connected to a rectal temperature probe. Arterial blood pressure was monitored with a miniature pressure transducer (Statham model P-37B, Gould Inc., Oxnard, CA 93030). Electrocardiac activity from implanted electrodes (see section on Hydrogen Clearance Method), blood

pressure, and body temperature were continuously recorded on a polygraph (Grass Instrument Co., Quincy, MA 02169). The electrocorticogram (ECoG) was subjected to spectral analysis of its component frequencies on line by fast Fourier transform (FFT).

C. Measurement of Cerebral Blood Flow by Hydrogen Clearance:

Epoxy-coated (epoxy resin, A-M Systems, Inc., Everett, Washington 98204) platinum-10% iridium electrodes (5 mil diameter, Engelhard, Carteret, NJ 07008) with an exposed 1-mm tip were stereotactically implanted into the anterior thalamus, the posterior hippocampus, and in selected animals also in the lateral and medial neocortex of both cerebral hemispheres. The pin connectors of the electrodes were embedded in methylmethacrylate cement to form a damage-resistant cap. Electrodes were polarized at +650 mV during experiments. After saturation of the brain to a breathing mixture containing 7% hydrogen, the hydrogen was turned off and the descent of the hydrogen-induced potential was recorded. Regional cerebral blood flow was calculated as the product of 0.693 and the time in minutes for the clearance curve of the hydrogen to decline halfway from its maximum during tissue saturation to the zero potential indicating complete washout of the hydrogen indicator. Additional details of the method are available elsewhere⁷.

D. Measurement of Cerebral Blood Flow by ¹⁴C-Iodoantipyrine Autoradiography:

Over the final 60 sec of the experiment, each rat received an intravenous infusion of 40 uCi of 4-(N-methyl-¹⁴C), iodoantipyrine (specific activity 54 uCi/mol, New England Nuclear, Boston, MA 02118) in normal saline delivered at a rate of 17 uL/sec by a syringe pump (Sage model 355, Orion Research, Cambridge, MA 02139)⁸. Arterial blood samples were collected at time zero and then at precisely 5-sec intervals during isotope infusion. At 1 min the circulation was arrested by decapitation. The brain was quickly

removed and frozen in isopentane chilled to -60°C . After warming to -18°C the brain was cut in 20- μm sections that were applied to glass cover slips, dried at 60°C and mounted sequentially on cardboard with a series of calibrated carbon-14 polymethylmethacrylate standards (Amersham, Arlington Heights, IL 60005). The sections were placed against a sheet of x-ray film (Kodak SB-5) in a cassette for an exposure of 4 d before developing as recommended by the manufacturer. The arterial blood samples were pipetted in 50- μL aliquots into scintillation vials into which were added hydrogen peroxide (30%, 250 μL), isopropanol (250 μL), and a tissue solubilizer (500 μL , Scintigest[®], Fisher Scientific). After dissolution of the blood by incubating at 60°C for 1 hr, 10 mL of scintillator were introduced into each vial after standing overnight at 4°C , the vials were counted for 10 min in a liquid scintillating counter (Packard TriCarb). The sequence of arterial blood counts was entered into a computer employing a program from Dr. L. Sokoloff⁸, and the regional cerebral blood flows of various gray and white matter structures were calculated on line with optical density readings determined by a microdensitometer (model DR-2H, Gamma Scientific, San Diego, CA 92123).

E. Factor VIII Preparation:

Twenty retired breeders served as blood donors. Under fluothane anesthesia, 9 mL of blood was drawn by cardiac puncture into 1 mL of sodium citrate (3.8%). The plasma was separated from the cells by centrifugation for 15 min at 5000 x G at 4°C . The plasma was spun a second time for 15 min at 10,000 X G at 4°C . Hemolyzed specimens were discarded. The cell-free plasma was brought to a pH of 6.88 by the addition of a 0.1 M acetic acid. Glycine was stirred into solution to attain a final concentration of 2.0 M, and the mixture was allowed to stand in an icebath for 1 hr to ensure complete precipitation. The precipitated material was collected by centrifugation for 15 min at 10,000 X G at 4°C . The proteinaceous button was gently dissolved in degassed ammonium

bicarbonate buffer (150 mM, pH 7.38) to one-tenth of the original volume of plasma. The solution was introduced into a siliconized chromatography column (K16/100, Pharmacia Fine Chemicals) packed with Bio-Gel A-50m (Bio-Rad Laboratories, Richmond, CA 94804) and eluted at room temperature with ammonium bicarbonate buffer and sodium azide (0.02%) at 15 mL/hr. The column effluent was collected in 3-mL fractions that were analyzed for protein content (Bio-Rad), fibrinogen (thrombin clotting time), and antihemophilic factor activity (one-stage activated partial thromboplastin time)⁹. The void-volume fractions preceding the emergence of fibrinogen were pooled and lyophilized.

F. Factor VIII Antiserum:

The lyophilized plasma fraction presumably containing rat factor-VIII-associated antigen was dissolved in 2 mL of normal saline. The solution was mixed with 4 mL of incomplete Freund's adjuvant and injected in small volumes subcutaneously in New Zealand albino rabbits. Inoculations were repeated every 2-3 wk. Rabbit blood was collected from the marginal vein of the ear. The clot was permitted to stand at 4°C overnight and the serum containing antibodies was pooled for later use. Sodium azide (0.02%) was added as a preservative.

G. Factor VIII Assays:

Antihemophilic factor activity was determined by the one-stage, activated partial thromboplastin time using human antihemophilic substrate plasma⁹. Attempts were also made to establish assays for factor-VIII-associated antigen by quantitative immunoelectrophoresis¹⁰ and for vonWillebrand factor by ristocetin agglutination of fixed, frozen and fresh, washed platelets¹¹.

H. Selection of a Model of Focal Cerebral Ischemia:

1. Background. Despite a small decline in blood flow through the ipsilateral cerebral hemisphere, ligation of a single carotid artery in the rat is ordinarily without metabolic or electrophysiological effects¹². However, some variability in this regard is encountered among different strains¹³. When combined with hypoxia, a single carotid ligation will produce ipsilateral ischemia with infarction in the rat¹⁴. This model of hypoxic-ischemic encephalopathy is beset by two major limitations: (1) high mortality during hypoxia owing to cardiovascular instability¹⁵ and (2) wide distribution of neuronal injury extending far beyond the zone of maximal ischemia¹⁶. Accordingly, three other models of focal ischemia were screened for their applicability to this project. These models included (a) carotid ligation in the spontaneously hypertensive rat, (b) ligation of the middle cerebral artery, and (c) selective air embolism into the internal carotid artery.

2. Cerebral Ischemia in the Spontaneously Hypertensive Rat. Unlike their normotensive counterparts, rats selectively inbred for the trait of spontaneous arterial hypertension develop a form of focal ischemia after ligation of the common carotid artery¹⁷. Animals of this Wistar-derived strain (Charles River Farms) were anesthetized with fluothane and oxygen. Electrodes were implanted in the anterior thalamus and posterior hippocampus bilaterally for monitoring regional cerebral blood flow by the hydrogen-clearance technique. Snare ligatures (9x0 nylon, Ethicon, Sommerville, NJ 08876) were placed around each common carotid artery and passed through the postauricular skin via a polyethylene catheter. Animals were restrained in a loose fitting plaster body jacket and permitted to awaken. After control measurements of cerebral blood flow were obtained, the carotid ligatures were pulled taut and clipped in place.

Pilot data demonstrated that the model of cerebral ischemia in the spontaneously hypertensive rat is subject to the certain limitations. First, the magnitude of the hypertension was variable and dependent upon the age of the rat such that younger

rats were often normotensive. The variability in the level of hypertension was probably responsible for the lack of reproducible cerebral ischemia. Affected animals were often lethargic or stuporous but unaffected animals showed no behavioral changes. A second limitation of the model was the fact that while the cerebral ischemia was focal, it was bilaterally symmetrical¹⁷ so and thus did not imitate the condition in man in whom a single vascular territory would typically be affected. As a consequence of these limitations, cerebral ischemia in the spontaneously hypertensive rat was not further investigated.

3. Middle Cerebral Artery Ligation. While transorbital ligation of the middle cerebral artery (MCA) is an established technique for producing focal ischemia in the monkey¹⁸, an analogous approach is not feasible in the rat owing to anatomical considerations. Nevertheless, a retro-orbital craniectomy provides access to the MCA just beyond its entry into the cranial cavity. After the dura mater was opened, a snare ligature (9x0 nylon, Ethicon) was passed under the MCA and carried to the surface through a polyethylene cannula glued to the side of the skull with alpha-cyanoacrylic cement. Contrary to a recent report¹⁹ that purported the effectiveness of this approach, ischemia of the appropriate portion of the brain was not found by hydrogen clearance measurement of cerebral blood flow, nor were behavioral changes of contralateral neurological dysfunction observed in animals subjected to MCA ligation. Consequently, this model was dismissed from further consideration.

4. Selective Air Embolism. Under the operating microscope, the first branch of the right external carotid artery (posterior occipital artery) was isolated and divided between two 6x0 silk ligatures leaving a 3-mm arterial stump. A 0.2-mm O.D. catheter fabricated from polyethylene tubing (PE-10, Clay Adams, Parsippany, NJ 07054) was introduced into the arterial stump and advanced retrograde just beyond the carotid bifurcation. The catheter tip was gently manipulated and directed by external version

into the internal carotid artery (ICA). The extracranial pterygopalatine branch of the ICA was ligated²⁰, and in later experiments the right eye was enucleated. The distal end of the ICA catheter was tunneled through the neck to emerge caudal to the left ear. The catheter was flushed with heparinized saline with care to prevent entry of air bubbles. Mean arterial blood pressure (MABP) was set by withdrawing or infusing blood from a femoral arterial catheter. Air in 2-, 5-, or 10-uL aliquots separated by saline was drawn into a 50-uL syringe (Gastight_R #1705, Hamilton Co., Reno, Nevada 89510). At the time of embolism, a bolus of air was rapidly injected into the ICA catheter. The ischemic effect was monitored by ECoG and by hydrogen-clearance measurements. At the conclusion of the ischemic interval (ordinarily 15 min) the BP was restored, and any acidosis was corrected by intravenous sodium bicarbonate and appropriate adjustment of the ventilator. Each experiment was terminated after 1 hr of follow-up observation. This model of focal ischemia was found most suitable of the three for these investigations.

J. Selection of a Model of Global Ischemia:

1. Background: An ample collateral circulation in the rat prevents cerebral ischemia when both common carotid arteries are occluded, so long as blood pressure remains normal²¹. When combined with prior electrocoagulation of the vertebral arteries, bilateral ligation of the common carotids results in global ischemia of the brain though the lower brainstem is spared²². Another method for producing even more effective ischemia of the central neuraxis is by elevation of the pressure of the CSF above the arterial blood pressure²³. Both of these approaches were studied for their usefulness as models of global ischemia.

2. CSF Compression Ischemia. Under fluothane anesthesia animals underwent a midthoracic cervical incision extended into the midoccipital region. The neck muscles were divided in the midline and detached at their paramedian insertions from the

occipital bone. A burrhole was drilled in the midline of the occipital bone, and the underlying dura mater was opened. A 5-mm blunt 20-gauge needle was wedged into the hole, and its base was sealed with alpha-cyanoacrylic cement. The needle was attached to a piece of plastic tubing (PE-90, Clay Adams) that was brought to the surface. The needle was embedded in methylmethacrylate and anchored in place with small stainless steel screws in the occipital bone. To induce ischemia, artificial CSF (Elliott's solution B) was infused through the catheter at rates as high as 5 mL/min. Because of the high rates of infusion required for ischemia, a decline in body temperature occurred along with fluid overload, the latter manifested as pulmonary edema after several minutes. Injection of Evans blue into the subarachnoid space revealed extensive shunting of CSF into the venous system and thence to the right heart. Accordingly, rapidly developing fluid overload precluded use of this model of global cerebral ischemia for 10 or 15 min intervals. Dr. Bo Siesjo confirmed this limitation of the model (personal communication), and the technique could not be adapted successfully to the rat for the purposes of these investigations.

3. Four-Vessel Occlusion Model. The method of Pulsinelli and Brierley²² was employed in male, Sprague-Dawley Rats weighing 200-400 g. Under fluothane anesthesia, a middorsal incision in the neck exposed the nuchal muscles that were divided down to the atlanto-occipital junction. The alar foramina of the atlas were identified and a monopolar microelectrode was passed into each foramen in turn. The underlying vertebral artery was electrocoagulated and the foramina packed with bone wax. The tissues were closed in layers. Through a midventral incision in the neck, each common carotid artery was isolated and a nylon ligature (9x0, Ethicon) looped about it. The ligature was threaded through a fine polyethylene tube tunneled laterally through the neck to emerge postauricularly. The ends of the ligature were tied about a short piece of tubing. The animals were permitted to recover for at least 24 hr. At the time of cerebral ischemia, the carotid ligatures were tightened and held fast by aneurysm clips. After 15

min of ischemia, the ligatures were severed, and animals were monitored for a minimum of 60 min before a terminal (^{14}C)-iodoantipyrine blood flow study. This model of global ischemia was deemed suitable for these investigations.

K. Therapeutic Interventions in Focal and Global Ischemia:

The drugs used in these studies were indomethacin (MSD L-590, 226), ibuprofen (Upjohn U-18, 573), flurbiprofen (Upjohn U-27, 182), and prostacyclin (Upjohn U-5321A). Indomethacin (1 mg/mL) was dissolved in normal saline with sodium carbonate (0.3 mg/mL). Ibuprofen and flurbiprofen were dissolved in tris-buffered saline (100 mM NaCl, 50 mM THAM, pH 7.38) each in a concentration of 10 mg/mL. Prostacyclin (100 $\mu\text{g/mL}$) was also dissolved in tris-buffered saline and kept chilled throughout the infusion. Heterologous antiserum was dialyzed against saline for 48 hr before infusion to remove sodium azide.

With the goal in mind of preventing postischemic hypoperfusion of the brain and thereby enhancing neural metabolism, the following treatment protocols were applied to the two models of ischemia:

	<u>Therapy</u>	<u>Dose</u>	<u>Route</u>
1.	Indomethacin	1, 2, 4, 10 mg/kg	intravenous
2.	Ibuprofen	30 mg/kg	intravenous
3.	Flurbiprofen	15 mg/kg	intravenous
4.	Prostacyclin plus Indomethacin	100 ng/ μL q.s. 4 or 10 mg/kg	intravenous, intracarotid intravenous
5.	Prostacyclin plus Ibuprofen	100 ng/ μL q.s. 30 mg/kg	intravenous intravenous
6.	Glass-wool filtration	30 min	arteriovenous
7.	Heterologous antiserum	5 mL	intravenous

Drugs 1-3 were administered over 5 min approximately 30-60 min before ischemia. Drug combinations 4 and 5 were given after ischemia, the indomethacin and ibuprofen immediately after the ischemic interval and the prostacyclin infusion commencing 5 min after ischemia. The prostacyclin was delivered by pump at the maximal rate (up to 10 ug/min) compatible with maintenance of an acceptable MABP (over 75 Torr). Glass-wool filtration over 30 min was carried out by shunting arterial blood through a 5-mL plastic chamber filled with lightly packed glass wool in saline. The blood returned via the femoral vein. Filters were changed at 10-min intervals.

L. Data Collection and Analysis:

Animals were discarded if blood pressure and arterial blood gases could not be maintained within acceptable limits or if CBF measurements fell below 50 mL/100 g/min before drugs or changes in blood pressure. Polarographically determined CBF's represent an aggregate value for cerebral gray matter. Each was calculated as the average of all measurements from all electrodes in FVO animals and in AE animals before injection of air. After embolization, each hemisphere was averaged separately. Every autoradiographically determined CBF represents the mean of 8 to 12 optical density measurements from each region. Statistical comparisons were carried out by Student's t-test employing a separate, untreated control group or the initial measurements in a given group before treatment.

IV.

RESULTS

A. Normative Data.

Values for local CBF were determined in nonischemic animals and compared with accepted figures. Polarographic data fell within established ranges for this laboratory, and autoradiographic data agreed favorably with those of Sakurada and coworkers (Table 1, Section 1A). Indomethacin (4 mg/kg) produced a global 10-20% reduction in CBF although the differences did not achieve statistical significance (Table 1, Section 1B). (This diminution of CBF was approximately half that reported by Dahlgren et al.²⁴ who gave indomethacin in a dose of 10 mg/kg.) In contrast, the potent vasodilator prostacyclin produced no consistent changes in CBF despite continuous intracarotid infusion in doses as high as 10 ug/min for as long as 2 hr.

B. Focal Ischemia Data in AE Model.

Measurement of local CBF by autoradiography showed that a 5-uL bolus of air injected into the internal carotid artery resulted in a perfusion defect chiefly in the distribution of the ipsilateral middle cerebral artery. Blood flow was typically interrupted throughout the cerebral hemisphere with variable involvement of the ipsilateral midbrain and parasagittal region. Immediately after embolization polarographic measurements typically revealed transient arrest of CBF lasting seconds to a couple of minutes, most pronounced in the neocortex and least in the posterior hippocampus. Correlation between MABP and the time that intravascular air resided in the vasculature was poor. Among untreated animals hydrogen-clearance data indicated that in 5 electrodes showing major postischemic hyperemia (>325 mL/100 g/min), 4 of the 5 lay in the nonembolized hemisphere and 4 of the 5 were in the neocortex. At 1 hr after ischemia the embolized hemisphere had uniformly lower CBF though the left-right differences did not reach

statistical significance (Table 1, Section IIA). In animals treated with indomethacin alone, indomethacin plus prostacyclin, or with flurbiprofen, serial CBF in the embolized hemisphere disclosed a highly significant diminution in the flows during hypotension with the first two regimens and at 1 hr after ischemia in all three (Table 2). The maximal postischemic flow was also decreased by all three treatments but without statistical significance (Table 2). At 1 hr after ischemia, local CBF was also consistently lower on the embolized side by autoradiographic determination though statistical significance was not achieved owing to large standard errors of the mean (Table 1, Section 2A). Indomethacin (4 mg/kg) generally lowered flow on the nonembolized and even more so on the embolized side, again without achieving statistical significance (Table 1, Section 2B). Glass-wool filtration produced results resembling those with indomethacin (Table 1, Section 2C).

In a separate study of the effect of serial microemboli (2 μ L) delivered into one internal carotid artery, it was discovered that each embolus markedly but transiently disrupted blood flow in the ipsilateral hemisphere. However, blood flow recovered but at a rate lower than that before the previous embolus (Table 3). This stepwise deterioration of CBF manifested an inverse relationship with the magnitude of MABP, the deterioration being fastest and requiring the least total air at 75 torr and slowest and requiring the most total air at 150 torr (Table 3). Deterioration of CBF at MABP of 100 and 125 torr lay between the two extremes. Repeated microemboli, regardless of the prevailing blood pressure, decreased CBF to within the narrow range of 25-35 mL/100 g/min (Table 3). Additional air did not lower the CBF further. The cumulative volume of air necessary to decrease the CBF highly significantly from the initial, control value was 6 μ L at 75 torr, 8 μ L at 100 and 125 torr, and 10 μ L at 150 torr (Table 3).

C. Global Ischemia in the FVO Model.

When mean arterial blood pressure remained in the normal range (126 ± 5 torr), measurements of rCBF in FVO animals disclosed levels of flow exceeding that required to produce high-grade ischemia, viz., approximately 15 mL/100 g/min (Table 4). An occasional autoradiographic study during the period of arterial occlusion showed relatively normal perfusion throughout the brain, and serial hydrogen-clearance determinations revealed low-normal rCBF. Because of such variability in the magnitude of ischemia, systemic arterial hypotension was combined with FVO to enhance the ischemic effect of vascular ligation. Hypotension at a level of 50 torr lowered polarographic CBF to less than 10 mL/100 g/min during ischemia and flows approaching zero were sometimes encountered (Table 4), while zero flow was never seen in normotensive animals. Autoradiography showed diminished or low-normal flow in the brainstem during ischemia while the hemispheres had no detectable flow (Table 4). During hypotension the arterial tension of carbon dioxide often declined and acidosis sometimes appeared during the postischemic period of recirculation.

Postischemic patterns of CBF differed between normo- and hypotensive control groups in that the magnitude of postischemic hyperemia was blunted in the hypotensive animals. Normotensive animals followed a predictable course in the postischemic period (Table 5). Maximal hyperemia occurred approximately 5 min after restoration of carotid flow, and the CBF returned to control values by 20-30 min into recirculation and remained within a narrow and slightly subnormal range for up to 120 min. Animals pretreated with indomethacin (4 mg/kg) evidenced a blunted hyperemic response, and the values of CBF fell to narrow range somewhat below that of control animals for up to 120 min of follow-up (Table 5). Postischemic hyperemia was even more impressively suppressed in animals pretreated with ibuprofen and infused with prostacyclin during the postischemic interval (Table 5). The addition of hypotension to ischemia

by FVO, as noted above, per se decreased the hyperemia and thus not surprisingly eliminated differences in postischemic CBF between treated and untreated animals as measured by hydrogen clearance. Indomethacin (10 mg/kg) did produce consistently lower rCBF 60 min after hypotensive ischemia than those in untreated animals but without statistical significance (Table 1, Section IIIB).

D. Factor VIII Results.

Only the assay for antihemophilic factor was successfully established for the rat. Rat plasma was remarkably refractory to ristocetin-induced clumping of fresh or fixed platelets from the rat and man. This difficulty has been observed by others who found that extremely high concentrations of ristocetin were required for a desired effect on platelets²⁵. Similarly, raising monospecific antiserum against presumed factor VIII-associated antigen proved an elusive goal. Plasma fractions known to harbor the antigenic activity in other species evoked production of several different antibodies as demonstrated by Ouchterlony gel diffusion and two-dimensional electrophoretic assays. In related work, plasma from several monkeys undergoing middle-cerebral artery ligation by the transorbital technique¹⁸ were studied. Measurements of antihemophilic and factor VIII antigenic activities failed to reveal any consistent changes brought about by reversible focal ischemia.

E. Cerebral Metabolic Changes in AE and FVO Animals.

As part of a pilot project the brains of animals subjected to 15 min of AE or FVO ischemia were frozen in liquid nitrogen and assayed for energy-related metabolites by Dr. Karl Conger (Department of Pathology, UAB). Global ischemia resulted in exhaustion of high-energy phosphate compounds and severe depletion of most intermediary carbohydrate compounds (Table 7). Lactate was elevated in the global model and

to a lesser degree in the focal model. However, possibly owing to the transient nature of air emboli, high-energy phosphates were normal. Surprisingly, the intermediary-carbohydrate metabolites were elevated not only in the embolized hemisphere but also in the nonembolized hemisphere and brainstem (Table 7).

V.

DISCUSSION

A series of investigations of canine cerebral ischemia by CSF compression and by air embolism has revealed a consistent pattern of enhanced postischemic perfusion with such diverse therapies as glass-wool filtration of the blood^{1, 4} and administration of certain drugs^{3, 5}. In view of the observations in man²⁶ and in the gerbil²⁷ that the magnitude of CBF immediately after ischemia correlates with a favorable neurologic outcome, promising therapeutic interventions may have application in the operational setting of naval medicine. Victims of cerebral aeroembolism, near-drowning or cardiopulmonary arrest are potential beneficiaries of these newer modes of treatment. Consequently, the present studies were undertaken in the rat to evaluate the cited work in the dog.

In selecting models of focal and global ischemia of the brain it was essential that the severity and duration of the ischemia insult be a reproducible and sufficient test of any proposed therapies. Selective air embolization and combined occlusion of the carotid and vertebral arteries with normal MABP led to unacceptable variability in local CBF oftentimes exceeding the ischemic threshold (approximately 15-20 mL/100 g/min). To improve the efficacy of each model, systemic arterial hypotension (MABP of 50 torr) was added during the ischemic interval. Nevertheless, a price was paid for introducing hypotension, viz., hypocapnia during ischemia, transient acidosis after ischemia, and even modification of the pattern of postischemic reperfusion. In the AE model the level of blood pressure was not related to the duration of decreased or arrested CBF after a 5-uL embolus, presumably the time that the intravascular air obstructs the microcirculation. On the other hand, blood pressure was found to be inversely related to the rate of deterioration of CBF that accompanied serial microemboli of air. These two conflicting observations suggest that, while tiny air bubbles pass through the capillary network, their transitory presence triggers a residual deterioration of microperfusion that is worse the

lower the MABP. Such deterioration in the wake of cerebral aeroembolism has been suspected on both theoretical and clinical grounds²⁸, but these experiments are the first to demonstrate its existence under controlled conditions.

Contrary to the original hypotheses of this project, nonsteroidal anti-inflammatory drugs with or without prostacyclin failed to enhance CBF after focal or global ischemia of the rat brain. Indeed, these drugs exerted a contrary effect by lowering or eliminating the usual hyperemia that follows upon reversible ischemia. Glass-wool filtration of the blood produced a similar response. Why the dog should manifest a contrasting result is not entirely clear. Although differences in experimental design could account for the conflicting results, it seems more likely that the contradictory observations reflect fundamental differences between species. Indeed, the findings in the rat are consonant with other work in the rat²⁴, baboon²⁹, and cat³⁰ whereby indomethacin decreased respectively the CBF in the resting state, CO₂-induced cerebral vasodilatation, and the hyperemic response after brief cerebral ischemia. Accordingly, that the dog responds idiosyncratically to drugs that influence prostaglandin-modulation of the cerebral microcirculation is an ineluctable deduction.

Preliminary examination of the metabolic profile of unilateral cerebral air embolism disclosed raised levels of most intermediary carbohydrate compounds, specifically including glucose and glucose-6-phosphate. Air embolization is known to alter the blood-brain barrier^{20, 31}, but it also elicits increased glucose utilization shown by 2-deoxyglucose autoradiography. This latter hypermetabolic state is a bilateral phenomenon³¹ and supports the findings of the pilot studies reported herein. The significance of this dysmetabolism is unclear.

Therapeutic implications from this work include experimental evidence to recommend (1) raising arterial BP to minimize microcirculatory deterioration during microaeroembolism of the brain and (2) administering nonsteroidal anti-inflammatory agents to

moderate high flow states in the brain when excessive flow might prove detrimental (e.g. certain vascular "steal" syndromes). Glass-wool filtration of blood might be similarly applied once its safety has been verified. The role of factor VIII in the pathogenesis of postischemic hypoperfusion of the brain was not clarified by this investigation.

VI.

CONCLUSIONS

- (1) In a focal (selective air embolism) and a global (four-vessel occlusion) model of reversible cerebral ischemia in the rat, nonsteroidal anti-inflammatory drugs (indomethacin, ibuprofen, flurbiprofen) blunt the magnitude of hyperemia following restoration of flow through the brain.
- (2) Prostacyclin given by intracarotid infusion exerts no effect on basal cerebral blood flow, but the compound combined with prostagland-synthetase inhibitors potentiates the depression of postischemic blood flow.
- (3) Ex-vivo circulation of blood through a glass-wool-filled chamber before ischemia eliminates the hyperemic response after ischemia.
- (4) Arterial hypotension intensifies cerebral ischemia in both focal and global models, and it diminishes the magnitude of postischemic hyperemia.
- (5) Serial microemboli of air result in a stepwise deterioration of local cerebral blood flow not explained by stationary intravascular air. The blood flow declines to a plateau of 25-35 mL/100g/min; however, the cumulative volume of air required to reach the plateau is inversely proportional to the initial MABP.
- (6) Unilateral cerebral air embolization stimulates production of intermediary carbohydrate metabolites throughout the brain and tends to increase blood flow in the nonembolized hemisphere, especially in the neocortex.

TABLE 1: CBF IN SELECTED REGIONS OF BRAIN ONE HOUR AFTER ISCHEMIA

		n	FC	PC	OL	OC	CP	HC	TH	CO	MB	PO
<u>I. CONTROL (No Ischemia & Normotension)</u>												
A. Untreated												
		6	139.1 +16.8	169.5 +19.5	119.8 +7.6	172.4 +24.4	141.2 +13.5	103.1 +9.8	160.7 +8.3	84.2 +8.2	128.1 +12.9	123.2 +8.7
B. Indomethacin (4 mg/kg)												
		5	118.6 +14.8	125.5 +6.8	105.9 +11.9	133.9 +15.1	122.2 +9.1	84.4 +4.9	133.2 +11.9	67.5 +8.2	107.7 +10.3	109.7 +10.0
<u>II. AIR EMBOLISM (Hypotension)</u>												
A. Untreated:												
	Nonembolized side	5	99.4 +28.9	111.8 +38.5	120.9 +33.3	141.9 +40.4	144.1 +43.5	100.3 +8.5	179.0 +59.6	46.8 +10.4	132.9 +31.1	139.4 +28.9
	Embolic side		96.2 +6.8	94.2 +14.3	100.6 +16.2	104.3 +11.7	82.6 +25.1	61.0 +8.6	98.2 +27.8	42.4 +7.0	121.1 +35.1	146.9 +34.8
B. Indomethacin (4 mg/kg):												
	Nonembolized side	5	88.5 +14.1	119.4 +29.1	105.4 +5.1	110.3 +20.1	103.9 +5.6	85.8 +9.6	127.4 +7.1	45.7 +11.3	101.2 +2.6	141.4 +8.9
	Embolic side		108.8 +38.8	83.7 +18.8	103.0 +16.0	90.6 +22.0	86.6 +11.6	76.7 +12.1	84.6 +11.6	31.0 +4.2	95.8 +11.8	139.9 +13.6
C. Glasswool filtration:												
	Nonembolized side	3	113.2 +16.6	96.1 +8.3	98.0 +14.8	62.8 +10.3	102.1 +26.1	93.1 +22.3	145.9 +27.0	29.2 +10.3	97.8 +18.5	93.6 +19.3
	Embolic side		89.9 +13.2	43.6 +8.8	110.9 +25.6	51.1 +10.8	90.4 +27.9	69.9 +17.8	112.7 +24.6	14.7 +5.6	93.3 +16.7	94.7 +16.7
<u>III. FOUR-VESSEL OCCLUSION (Hypotension)</u>												
A. Untreated												
		6	98.5 +35.4	52.8 +12.2	53.3 +12.6	56.0 +24.8	61.3 +15.1	42.8 +9.1	62.4 +6.4	17.0 +2.3	66.5 +8.4	77.3 +14.0
B. Indomethacin (10 mg/kg)												
		5	40.6 +7.8	42.8 +12.5	46.3 +8.0	36.1 +7.7	45.0 +7.1	30.7 +4.5	56.2 +7.1	17.3 +5.3	59.6 +12.3	66.4 +14.8

Autoradiographically measured CBF in ml/100 g/min (mean + S.E.M.). FC=frontal cortex, PC=parietal cortex, OL=olfactory cortex, OC=occipital cortex, CP=caudate-putamen, HC=hippocampus, TH=thalamus, CO=centrum ovale, MB=midbrain, PO=pons.

TABLE 2: CBF DURING AIR EMBOLISM WITH HYPOTENSION (MABP=50 TORR).

	NO TREATMENT	INDOMETHACIN	INDOMETHACIN/ PGI ₂	FLURBIPROFEN
BASELINE	115.2 $\pm$ 6.1 (5)	109.3 $\pm$ 21.3 (6)	122.2 $\pm$ 16.9 (5)	108.7 $\pm$ 2.1 (3)
POST DRUG	-----	93.5 $\pm$ 15.3 (6)	-----	77.3 $\pm$ 9.6 (3)
HYPOTENSION	80.6 $\pm$ 11.0 (5)	60.1 $\pm$ 5.3 (6)*	62.5 $\pm$ 6.7 (5)*	79.0 $\pm$ 13.8 (3)
POSTISCHEMIC MAXIMUM	162.9 $\pm$ 23.0 (5)	124.2 $\pm$ 13.0 (5)	101.2 $\pm$ 22.5 (5)	87.7 $\pm$ 13.0 (3)
1-HR POST ISCHEMIA	94.0 $\pm$ 3.3 (5)	55.6 $\pm$ 8.2 (5)*	58.9 $\pm$ 8.0 (5)*	47.6 $\pm$ 12.6 (3)*

CBF in mL/100 g/min; mean $\pm$ S.E.M.; n in parentheses.

*p < 0.01.

TABLE 3: EFFECT OF SERIAL AIR MICROEMBOLI ON CBF AT DIFFERENT MABP

CUMULATIVE DOSE OF AIR (uL)	MEAN ARTERIAL BLOOD PRESSURE (Torr)			
	150 (n=2)	125 (n=3)	100 (n=3)	75 (n=4)
0	103 $\pm$ 12	77 $\pm$ 5	95 $\pm$ 5	77 $\pm$ 9
2	113 $\pm$ 1	75 $\pm$ 2	83 $\pm$ 20	63 $\pm$ 5
4	80 $\pm$ 18	64 $\pm$ 5	55 $\pm$ 12 *	40 $\pm$ 2 *
6	62 $\pm$ 6	54 $\pm$ 5 *	49 $\pm$ 12 *	30 $\pm$ 3 **
8	50 $\pm$ 4 *	38 $\pm$ 8 **	44 $\pm$ 13 **	40 $\pm$ 8
10	41 $\pm$ 3 **	30 $\pm$ 10	54 $\pm$ 20	32 $\pm$ 4
12	42 $\pm$ 7	30 $\pm$ 6	35 $\pm$ 12	34 $\pm$ 1
14	35 $\pm$ 4	26 $\pm$ 0	32 $\pm$ 8	33 $\pm$ 3
16	32 $\pm$ 2	28 $\pm$ 0	24 $\pm$ 0	35 $\pm$ 3

Polarographically measured CBF in mL/100 g/min; *p<0.05, **p<0.01;
 Statistical comparisons are made with the control value of CBF at each MABP.

TABLE 4: COMPARISON OF CEREBRAL ISCHEMIA IN NORMO- AND HYPOTENSIVE RATS DURING FOUR-VESSEL OCCLUSION.

	n	FC	PC	OL	OC	CP	HC	TH	CO	MB	PO
NORMOTENSIVE ANIMALS	2	57.7 +24.5	66.0 +12.2	47.8 +10.2	46.5 +15.2	56.9 +18.2	46.5 +5.0	69.2 +2.2	32.1 +20.5	90.9 +13.2	95.5 +13.5
HYPOTENSIVE ANIMALS	3	3.2 +0.9	3.8 +0.8	5.1 +1.5	3.7 +0.6	3.6 +0.8	4.2 +1.0	4.3 +0.3	2.9 +1.5	9.7 +5.0	18.7 +6.9
			*	**	**	**	*	*		*	**

Autoradiographically measured CBF in ml/100 g/min (mean + S.E.M.); FC=frontal cortex, PC=parietal cortex, OL=olfactory cortex, OC=occipital cortex, CP=caudate putamen, HC=hippocampus, TH=thalamus, CO=centrum ovale, MB=midbrain, PO=pons.

*p < 0.01, **p < 0.05.

TABLE 5: CBF DURING FOUR-VESSEL OCCLUSION WITH NORMAL BP.

	NO TREATMENT	INDOMETHACIN	IBUPROFEN/ PGI_2
BASELINE	84.4 ± 13.8 (6)	93.0 ± 15.9 (4)	$56. \pm 3.1$ (5)
ISCHEMIA	13.0 ± 2.7 (6)	12.0 ± 2.0 (6)	11.5 ± 2.6 (5)
POSTISCHEMIC MAXIMUM	378.0 ± 72.6 (6)	168.2 ± 33.2 (6)*	63.0 ± 15.3 (5)**
1-HR POST ISCHEMIA	58.4 ± 10.8 (6)	59.1 ± 18.0 (6)	34.8 ± 3.5 (5)

Polarographically measured CBF in mL/100 g/min; mean $\pm$ S.E.M.; n in parentheses.

*p <0.05, **p <0.01.

TABLE 6: CBF DURING FOUR-VESSEL OCCLUSION
WITH HYPOTENSION (MABP=50 TORR).

	NO TREATMENT	INDOMETHACIN
BASELINE	95.3 $\pm$ 10.2 (7)	90.0 $\pm$ 5.9 (7)
HYPOTENSION	100.0 $\pm$ 16.2 (5)	78.7 $\pm$ 4.6 (5)*
ISCHEMIA	9.5 $\pm$ 2.7 (5)	7.2 $\pm$ 1.4 (7)*
POSTISCHEMIC MAXIMUM	251.6 $\pm$ 24.9 (5)	264.8 $\pm$ 26.1 (8)*
1-HR POST ISCHEMIA	104.0 $\pm$ 24.3 (5)	126.4 $\pm$ 28.3 (8)*

Polarographically measured CBF in mL/100 g/min; mean $\pm$ S.E.M.; n in parentheses.

*p < 0.05

TABLE 7: BIOCHEMICAL PROFILES AFTER FOCAL (AIR EMBOLISM) AND GLOBAL (FOUR-VESSEL OCCLUSION) ISCHEMIA IN THE RAT BRAIN

<u>COMPOUND</u>	CONTROL		FOCAL ISCHEMIA			GLOBAL ISCHEMIA	
	H	B	IH	CH	B	H	B
Glucose	1.0	1.0	1.4	1.5	2.6	0.2	9.0
Glucose-6-P	1.0	1.0	1.8	1.7	1.6	0.7	1.4
Fructose-6-P	1.0	1.0	2.4	2.2	1.8	0.9	1.5
-Ketoglutarate	1.0	1.0	1.2	1.3	1.4	0.1	0.5
Lactate	1.0	1.0	3.0	1.9	2.1	4.9	7.3
Pyruvate	1.0	1.0	1.6	1.6	2.0	0.5	1.3
ATP	1.0	1.0	0.9	1.1	1.5	0.0	0.2
Phosphocreatinine	1.0	1.0	1.0	1.0	1.6	0.1	0.2

*H=cerebral hemisphere; IH=ipsilateral (embolized) hemisphere; CH=contralateral hemisphere; B=brainstem/cerebellum. All values are relative to those of control group (n=5 for each group).

VIII.

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